

case, orf virus infection was suspected because of the patient's occupational exposure and clinical compatible skin lesions (e.g., single pustular lesion and erythema multiforme aspect on the rest of the body and the absence of systemic symptoms) (9); infection was diagnosed with positive parapoxvirus PCR test (3). However, an unusual recent case in Portugal involved monkeypox infection after a needle stick injury (10). The patient had a solitary pustular lesion of the finger, similar to our patient, but that lesion was painful, and the clinical picture was completed with the appearance of diffuse vesicles and systemic symptoms.

This case highlights the importance of collecting a careful history at the time of patient care, including collection of exposures to possible zoonoses. Those measures are warranted to avoid unnecessary isolation and treatment and to enable appropriate infection control measures.

C.C. and S.Z. were the major contributors in writing the manuscript and performing the literature review.

A.S.D. provided the pictures and the legend. A.F.R. and O.F. conducted the microbiologic study. T.K. revised the manuscript. Both lead authors have read and agreed to the published version of the manuscript. The data presented in this case study are available on request from the corresponding author.

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## About the Author

Dr. Zayet is a specialist in the Infectious Diseases Department of Nord Franche-Comté Hospital, Trevenans, France. His primary research interests focus on hepatitis and tuberculosis, especially in HIV-infected patients and COVID-19 patients.

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Address for correspondence: Souheil Zayet, Department of Infectious Disease, Nord Franche-Comté Hospital, 100 Route de Moval, 90400 Trevenans, France; email: souhail.zayet@gmail.com or souheil.zayet@hnfc.fr

## SARS-CoV-2 Molecular Evolutionary Dynamics in the Greater Accra Region, Ghana

Bright Adu,<sup>1</sup> Joseph H.K. Bonney,<sup>1</sup> Beverly Egyir, Isaac Darko Otchere, Prince Asare, Francis E. Dennis, Evelyn Yayra Bonney, Richard Akuffo, Ivy A. Asante, Evangeline Obodai, Selassie Kumordzie, Joyce Appiah-Kubi, Quaneeta Mohktar, Hilda Opoku Frempong, Franklin Asiedu-Bekoe, Mildred A. Adusei-Poku, James O. Aboagye, Bright Agbodzi, Clara Yeboah, Seyram B. Agbenyo, Peace O. Uche, Keren O. Attiku, Bernice Twenewaa Sekyere, Dennis Laryea, Kwame Buabeng, Helena Lampsey, Anita Ghansah, Dorothy Yeboah-Manu, Abraham K. Anang, William K. Ampofo, George B. Kyei, John K. Odoom

<sup>1</sup>These authors contributed equally to this article.

Author affiliations: Noguchi Memorial Institute for Medical Research, University of Ghana College of Health Sciences, Legon, Ghana (B. Adu, J.H.K. Bonney, B. Egyir, I.D. Otchere, P. Asare, F.E. Dennis, E.Y. Bonney, R. Akuffo, I.A. Asante, E. Obodai, S. Kumordzie, J. Appiah-Kubi, Q. Mohktar, H. Opoku Frempong, J.O. Aboagye, B. Agbodzi, C. Yeboah, S.B. Agbenyo, P.O. Uche, K.O. Attiku, B. Twenewaa Sekyere, D. Laryea, K. Buabeng, H. Lamptey, A. Ghansah, D. Yeboah-Manu, A.K. Anang, W.K. Ampofo, G.B. Kyei, J.K. Odoom); Ghana Health Service, Accra, Ghana (F. Asiedu-Bekoe); University of Ghana Medical School, Accra (M.A. Adusei-Poku); University of Ghana Medical Centre, Legon (G.B. Kyei)

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To assess dynamics of SARS-CoV-2 in Greater Accra Region, Ghana, we analyzed SARS-CoV-2 genomic sequences from persons in the community and returning from international travel. The Accra Metropolitan District was a major origin of virus spread to other districts and should be a primary focus for interventions against future infectious disease outbreaks.

The emergence of SARS-CoV-2 variants with superior transmissibility or immune evasion advantages may cause outbreaks and dominate transmission in a population (1). Thus, keeping track of the dynamics of variant transmissions in a population is crucial for developing timely and appropriate responses to outbreaks.

In Ghana, whereas the entire population experienced the COVID-19 pandemic, most infections were primarily recorded in the Greater Accra Region (GAR), the most densely populated region in Ghana with the smallest landmass (2). The genetic diversity of SARS-CoV-2 infections in Ghana during early (3) and recent (4) transmissions showed initial transmission driven by multiple lineages of the virus, after which the Alpha, Delta, and Omicron variants dominated. To gain information about the dynamics of SARS-CoV-2 spread within the GAR, the epicenter of the COVID-19 outbreak in Ghana, we performed a detailed analysis of variants.

We analyzed 1,163 SARS-CoV-2 genomic sequences from 834 community samples collected from 14 of the 21 districts in the GAR and 329 from returning international travelers (Table) during March 2020–February 2022. We extracted RNA from oro/nasopharyngeal swab samples of patients by using a QIAamp Viral RNA Mini Kit (QIAGEN, <https://www.qiagen.com>).

We prepared complementary DNA by using the LunaScript RT Super Mix Kit (New England Bio-Labs, <https://www.neb.com>). For amplicon generation, we used either the ARTIC nCoV-2019 version 3

**Table.** Distribution of SARS-CoV-2 sequences analyzed by district of Ghana and origin of international travelers

Origin of travelers	Sequences, no. (%)
Ghana, n = 834	
Accra Metropolitan District	421 (50.5)
Ashaiman Municipal	1 (0.1)
Adenta Municipal	41 (4.9)
Ga East	19 (2.3)
Ga Central	8 (1.0)
Ga South	6 (0.7)
Ga West	21 (2.5)
Kpone Katamanso	1 (0.1)
La-Dade Kotopon	21 (2.5)
La-Nkwantanang Madina	9 (1.1)
Ledzokuku Krowor	6 (0.7)
Ningo Prampram	1 (0.1)
Shai Osudoku	12 (1.4)
Tema Municipal	25 (3.0)
Unnamed district*	242 (29.0)
World, n = 329	
Africa	159 (48.3)
Asia	85 (25.8)
Europe	57 (17.3)
North America	28 (8.5)

\*Samples from within the Greater Accra Region but with no clear indication of the specific district.

primers (Artic Network, <https://artic.network>) (batch 1 samples, collected before July 2021) or the Midnight RT PCR Expansion kit (Oxford Nanopore Technologies, <https://www.nanoporetech.com>) (batch 2 samples, collected after July 2021). We sequenced batch 1 samples on Illumina MiSeq after library preparation with an Illumina DNA prep kit (<https://www.illumina.com>) and batch 2 samples on GridION after library preparation with SQK-RBK110.96 kit (Oxford Nanopore Technologies).

For both batches of samples, we analyzed reads by using the ARTIC version 1.2 field bioinformatics pipeline (<https://github.com/artic-network/fieldbioinformatics>). We assigned Lineages by using Pangolin version 4.1.3 with pangolin-data version 1.17 (5).

For the phylogenetic analysis, we first aligned sequences in MAFFT version 7.490 (6). We inferred the maximum-likelihood tree topology of the variable positions with 1,000 bootstraps by using IQ-TREE version 2.0.7 (7) with the general time reversible nucleotide substitution model. We populated the maximum-likelihood tree with sampling dates by using TreeTime version 0.8.6 (8) and assuming a mean constant nucleotide substitutions per site per year rate of  $8.0 \times 10^{-4}$  (9) after excluding outlier sequences. We then rerooted the final dated tree with 936 sequences to the initial wild-type SARS-CoV-2 strain (GenBank accession no. NC\_045512.2) and visualized in R version 4.1.2 (<https://www.r-project.org>) by using ggtree version 3.2.1 and ggtreeExtra version 1.4.2 packages (10). For the import-export analysis, we labeled the internal nodes and external

leaves of the dated phylogeny with the location/district of sample origin by using TreeTime. We inferred the number of state changes from one location/district to another and time of event by using a python script developed by Wilkinson Lab ([https://github.com/CERI-KRISP/africa-covid19-genomics/tree/main/python\\_scripts](https://github.com/CERI-KRISP/africa-covid19-genomics/tree/main/python_scripts)).

Of the 152,896 SARS-CoV-2 infections reported in Ghana by February 28, 2022, the GAR alone contributed 90,267 (59.04%) (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/29/4/22-1410-App1.pdf>). Of the 21 districts in the GAR, the Accra Metropolitan District (AMD) consistently contributed ~50% of reported SARS-CoV-2 infections in the region since the outbreak began in Ghana (<https://ghs.gov.gh/covid19/archive.php>). This finding mirrors our finding of 50.5% of sequences from the region being from the AMD (Table). Although all analyzed sequences (Appendix Table 2) came from the GAR, representative metadata for some samples were not indicated by all districts. Those districts were grouped as "Unnamed District" and accounted for 29% of the sequences, most of which were the Alpha variant (Appendix Figure 1).

Because different lineages have dominated SARS-CoV-2 transmission in Ghana at different periods, we categorized the data into the main SARS-CoV-2 variants (Alpha, Beta, Delta, Eta, Omicron, and others). From the phylogenetic analysis, the SARS-CoV-2 variants circulating in the districts of the GAR and those from returning international travelers resolved into 5 major clusters corresponding to defined categories (Appendix Figure 2, panel A). Sequences from the returning international travelers colocalized with the GAR samples, suggesting minimal divergence. We found that an estimated 77 SARS-CoV-2 variant introduction events occurred in the AMD, mainly from other parts of Africa and other districts (Appendix Figure 2, panel B). In contrast, there were an estimated 185 SARS-CoV-2 variant exportation events from the AMD, mainly to the other districts of the GAR and to relatively fewer to countries outside Ghana (Appendix Figure 2, panels C, D). Of those variant exportation events, 153 were to other districts in the GAR, making the AMD a prime district for targeted interventions aimed at reducing the spread of SARS-CoV-2 and other infectious pathogens.

In conclusion, SARS-CoV-2 genomic surveillance in the GAR of Ghana revealed the pattern of spread of variants among districts of the region, demonstrating the role of the AMD in the spread of SARS-CoV-2 in the GAR. We propose that the AMD should be a primary focus in public health interventions aimed at controlling SARS-CoV-2 and other future infectious disease outbreaks in the GAR.

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## About the Author

Dr. Adu is a senior research fellow at the Noguchi Memorial Institute for Medical Research of the University of Ghana and the coordinator for the Next Generation Sequencing Core Facility of the Institute. His research interests include pathogen genomics and immunology.

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Address for correspondence: Bright Adu, Department of Immunology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, PO Box LG 581, Legon, Ghana; email: badu@noguchi.ug.edu.gh

## Genomic Characterization of Respiratory Syncytial Virus during 2022–23 Outbreak, Washington, USA

Stephanie Goya, Jaydee Sereewit, Daniel Pfalmer, Tien V. Nguyen, Shah A.K. Mohamed Bakhsh, Elizabeth B. Sobolik, Alexander L. Greninger

Author affiliations: University of Washington, Seattle, Washington, USA (S. Goya, J. Sereewit, D. Pfalmer, T.V. Nguyen, S.A.K. Mohamed Bakhsh, E.B. Sobolik, A.L. Greninger); Fred Hutchinson Cancer Research Center, Seattle (A.L. Greninger)

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We sequenced 54 respiratory syncytial virus (RSV) genomes collected during 2021–22 and 2022–23 outbreaks in Washington, USA, to determine the origin of increased RSV cases. Detected RSV strains have been spreading for >10 years, suggesting a role for diminished population immunity from low RSV exposure during the COVID-19 pandemic.

**A**nnual seasonality of respiratory syncytial virus (RSV) in Washington, USA, has been limited primarily to late autumn and winter (1). However, an RSV outbreak was not detected during the 2020–21 season because of the COVID-19 pandemic. After lockdowns were relaxed in the summer of 2021, an early RSV season began in August (Figure, panel A). The 2022–23 outbreak also began earlier, but the number of RSV cases was unexpectedly higher than in 2021, alarming public health authorities and the general community (2).

Increased severity of the 2022–23 RSV outbreak might have been caused by diminished protective immunity in the population from prolonged low exposure to this virus (3). Furthermore, selective pressure because of low transmission in 2020 might have caused emergence of new viral strains with improved fitness. We evaluated whether RSV causing the 2022–23 outbreak had genomic characteristics different from strains from previous seasons.

We performed hybridization capture-based, metagenomic next-generation sequencing of 54 RSV genomes (14 RSV strains from 2021–22 and 40 from 2022–23) isolated during outbreaks in King County, Washington. In brief, we extracted virus RNA from excess nasal or nasopharyngeal swab specimens collected from persons seeking care at University of Washington Medicine COVID-19 collection sites, clinics, emergency rooms, and inpatient facilities who tested positive for RSV by PCR with a cycle threshold <30 (Table) (4). All persons were outpatients except for 2 hospitalized patients from 2021. For phylogenetic analyses, we downloaded complete genomes of RSV-A and RSV-B subtypes from GenBank and GISAID (<https://www.gisaid.org>) databases. We performed genome alignments by using MAFFT software (<https://mafft.cbrc.jp/alignment/software>) and constructed phylogenetic trees by using IQ-TREE (5) (Appendix, <https://wwwnc.cdc.gov/EID/article/29/4/22-1834-App1.pdf>).

Among sequenced specimens, we detected 1 RSV-A and 13 RSV-B subtypes from 2021–22 and 30 RSV-A and 10 RSV-B subtypes from 2022–23 (Table). We did not detect co-infections with other respiratory viruses (Appendix) or differences in subtype predominance by patient age group or sex during the 2022–23 outbreak ( $p>0.1$  by Fisher exact test). We genotyped the RSV G gene and found that 7 RSV-A sequences were GA2.3.5 and 24 were GA2.3.6b genotypes (both comprising ON1 strains), and all RSV-B sequences were the GB5.0.5.a genotype (BA strains) (6) (Appendix). We found that Washington RSV (WA-RSV) sequences were closely related to contemporary viruses by

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# SARS-CoV-2 Molecular Evolutionary Dynamics in the Greater Accra Region, Ghana

## Appendix

**Appendix Table 1.** SARS-CoV-2 infections in Ghana by region from March 2020 to February 28, 2022\*

Region	SARS-CoV-2 infections (count)	Percentage (%)
Greater Accra	90,267	59.04
Ashanti	22,292	14.58
Western	8,311	5.44
Eastern	7,032	4.60
Volta	5,954	3.89
Central	5,402	3.53
Bono East	2,966	1.94
Bono	2,332	1.53
Northern	1,863	1.22
Upper East	1,733	1.13
Ahafo	1,135	0.74
Western North	1,109	0.73
Oti	930	0.61
Upper West	895	0.59
North East	384	0.25
Savannah	291	0.19
Total	152,896	100.00

\*Data source. Ghana Health Service COVID-19 outbreak response management updates. (<https://www.ghs.gov.gh/covid19/archive.php>).

**Appendix Table 2.** GISAID accession numbers of SARS-CoV-2 genomic sequences used in the study

gisaid_epl_isl	gisaid_epl_isl	gisaid_epl_isl	gisaid_epl_isl	gisaid_epl_isl	gisaid_epl_isl	gisaid_epl_isl
Samples from the Greater Accra region						
EPI_ISL_422382	EPI_ISL_2508389	EPI_ISL_8065554	EPI_ISL_8065656	EPI_ISL_8065756	EPI_ISL_8065853	EPI_ISL_8065956
EPI_ISL_422384	EPI_ISL_2508390	EPI_ISL_8065555	EPI_ISL_8065655	EPI_ISL_8065757	EPI_ISL_8065856	EPI_ISL_8065958
EPI_ISL_422404	EPI_ISL_2508391	EPI_ISL_8065556	EPI_ISL_8065654	EPI_ISL_8065758	EPI_ISL_8065858	EPI_ISL_8065959
EPI_ISL_422387	EPI_ISL_2508392	EPI_ISL_8065562	EPI_ISL_8065657	EPI_ISL_8065750	EPI_ISL_8065859	EPI_ISL_8065960
EPI_ISL_422405	EPI_ISL_2508393	EPI_ISL_8065558	EPI_ISL_8065658	EPI_ISL_8065752	EPI_ISL_8065857	EPI_ISL_8065961
EPI_ISL_422390	EPI_ISL_2508394	EPI_ISL_8065559	EPI_ISL_8065659	EPI_ISL_8065761	EPI_ISL_8065862	EPI_ISL_8065962
EPI_ISL_422394	EPI_ISL_2508395	EPI_ISL_8065560	EPI_ISL_8065662	EPI_ISL_8065763	EPI_ISL_8065863	EPI_ISL_8065964
EPI_ISL_515083	EPI_ISL_2508396	EPI_ISL_8065561	EPI_ISL_8065664	EPI_ISL_8065768	EPI_ISL_8065865	EPI_ISL_8065963
EPI_ISL_515084	EPI_ISL_8065519	EPI_ISL_8065557	EPI_ISL_8065663	EPI_ISL_8065759	EPI_ISL_8065861	EPI_ISL_8065965
EPI_ISL_515085	EPI_ISL_8065520	EPI_ISL_8065563	EPI_ISL_8065661	EPI_ISL_8065762	EPI_ISL_8065864	EPI_ISL_8065966
EPI_ISL_515082	EPI_ISL_8065521	EPI_ISL_8065564	EPI_ISL_8065665	EPI_ISL_8065764	EPI_ISL_8065860	EPI_ISL_8065967
EPI_ISL_422397	EPI_ISL_8065524	EPI_ISL_8065565	EPI_ISL_8065666	EPI_ISL_8065765	EPI_ISL_8065866	EPI_ISL_8065970
EPI_ISL_422406	EPI_ISL_8065522	EPI_ISL_8065570	EPI_ISL_8065668	EPI_ISL_8065766	EPI_ISL_8065869	EPI_ISL_8065969
EPI_ISL_422398	EPI_ISL_8065517	EPI_ISL_8065566	EPI_ISL_8065669	EPI_ISL_8065767	EPI_ISL_8065870	EPI_ISL_8065968
EPI_ISL_422402	EPI_ISL_8065518	EPI_ISL_8065567	EPI_ISL_8065660	EPI_ISL_8065760	EPI_ISL_8065873	EPI_ISL_8065972
EPI_ISL_422403	EPI_ISL_8065523	EPI_ISL_8065568	EPI_ISL_8065667	EPI_ISL_8065769	EPI_ISL_8065867	EPI_ISL_8065971
EPI_ISL_422400	EPI_ISL_2361908	EPI_ISL_8065569	EPI_ISL_8065670	EPI_ISL_8065790	EPI_ISL_8065868	EPI_ISL_8065975
EPI_ISL_422399	EPI_ISL_2361909	EPI_ISL_8065572	EPI_ISL_8065671	EPI_ISL_8065773	EPI_ISL_8065872	EPI_ISL_8065974
EPI_ISL_422401	EPI_ISL_2376383	EPI_ISL_8065571	EPI_ISL_8065672	EPI_ISL_8065785	EPI_ISL_8065871	EPI_ISL_8065973
EPI_ISL_515103	EPI_ISL_2361910	EPI_ISL_8065577	EPI_ISL_8065673	EPI_ISL_8065786	EPI_ISL_8065876	EPI_ISL_8065977
EPI_ISL_515098	EPI_ISL_2508381	EPI_ISL_8065575	EPI_ISL_8065674	EPI_ISL_8065789	EPI_ISL_8065874	EPI_ISL_8065976
EPI_ISL_515100	EPI_ISL_2508382	EPI_ISL_8065573	EPI_ISL_8065675	EPI_ISL_8065795	EPI_ISL_8065877	EPI_ISL_8065978
EPI_ISL_515099	EPI_ISL_2508380	EPI_ISL_8065576	EPI_ISL_8065692	EPI_ISL_8065796	EPI_ISL_8065878	EPI_ISL_8065979
EPI_ISL_515101	EPI_ISL_2508383	EPI_ISL_8065574	EPI_ISL_8065702	EPI_ISL_8065779	EPI_ISL_8065875	EPI_ISL_8065980
EPI_ISL_515086	EPI_ISL_2508384	EPI_ISL_8065578	EPI_ISL_8065680	EPI_ISL_8065771	EPI_ISL_8065880	EPI_ISL_8065982
EPI_ISL_515087	EPI_ISL_2361911	EPI_ISL_8065580	EPI_ISL_8065691	EPI_ISL_8065772	EPI_ISL_8065879	EPI_ISL_8065981
EPI_ISL_515089	EPI_ISL_2361912	EPI_ISL_8065579	EPI_ISL_8065697	EPI_ISL_8065774	EPI_ISL_8065883	EPI_ISL_8065983
EPI_ISL_515088	EPI_ISL_2361913	EPI_ISL_8065581	EPI_ISL_8065698	EPI_ISL_8065775	EPI_ISL_8065881	EPI_ISL_8065984
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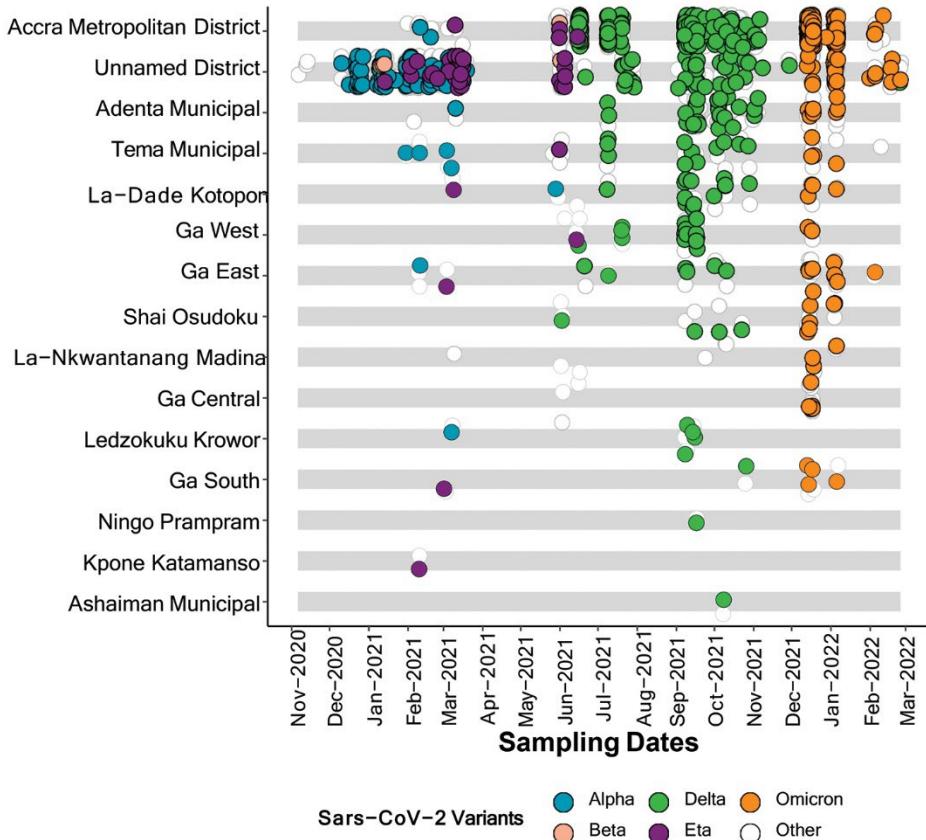
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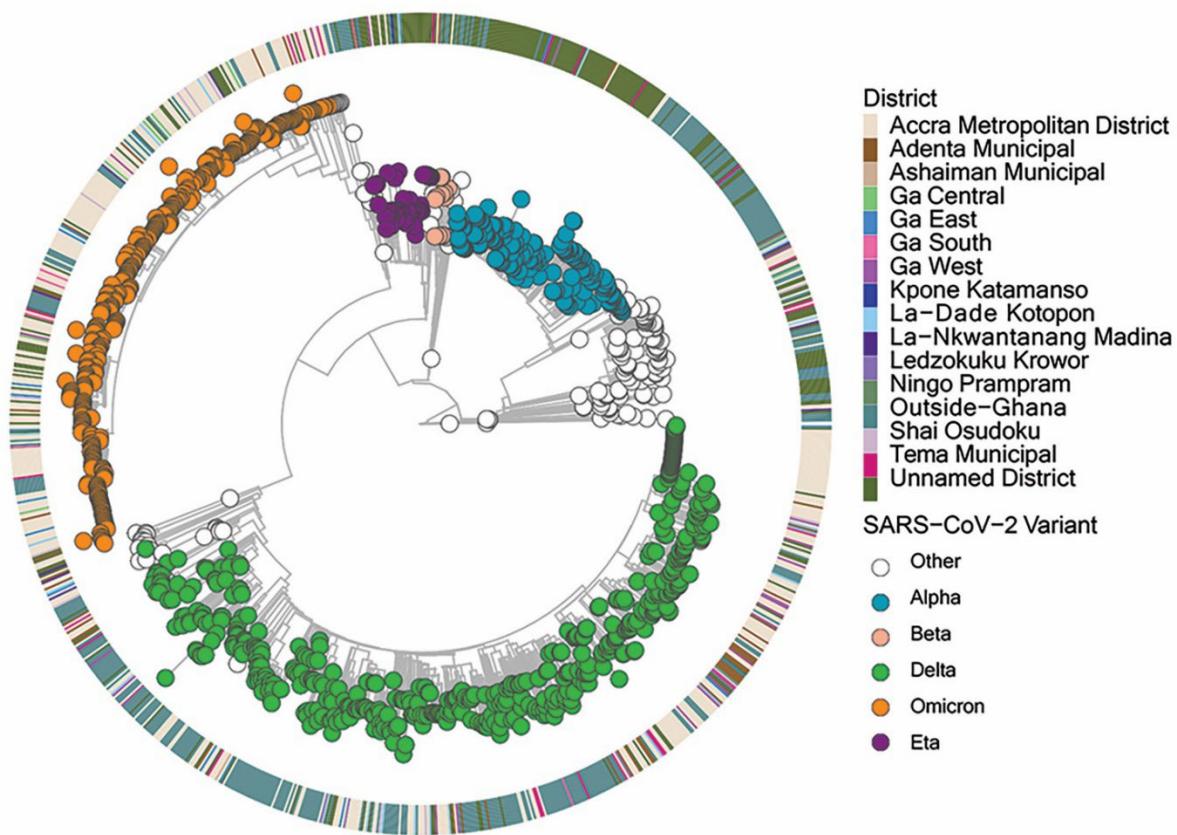
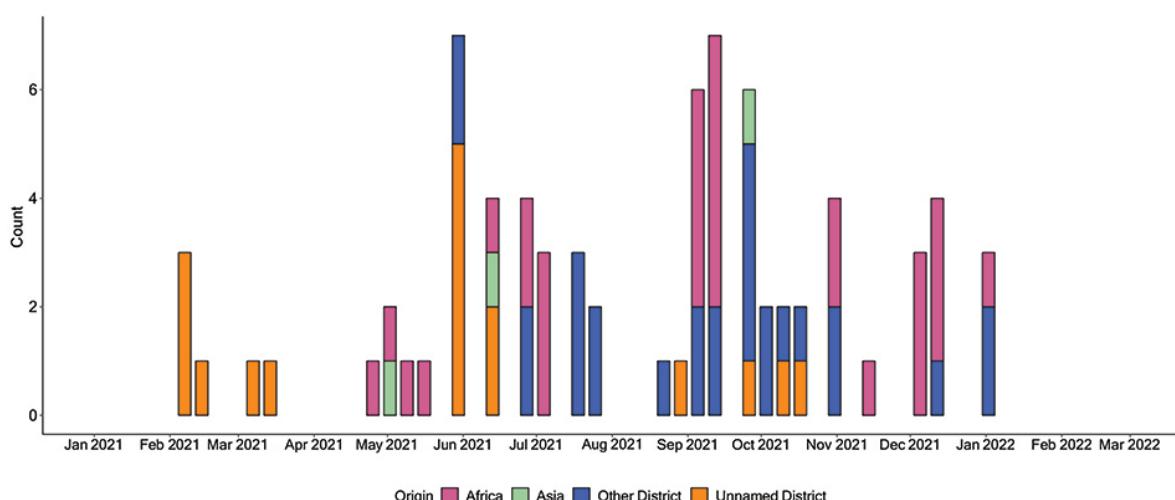
Samples from returning International travelers

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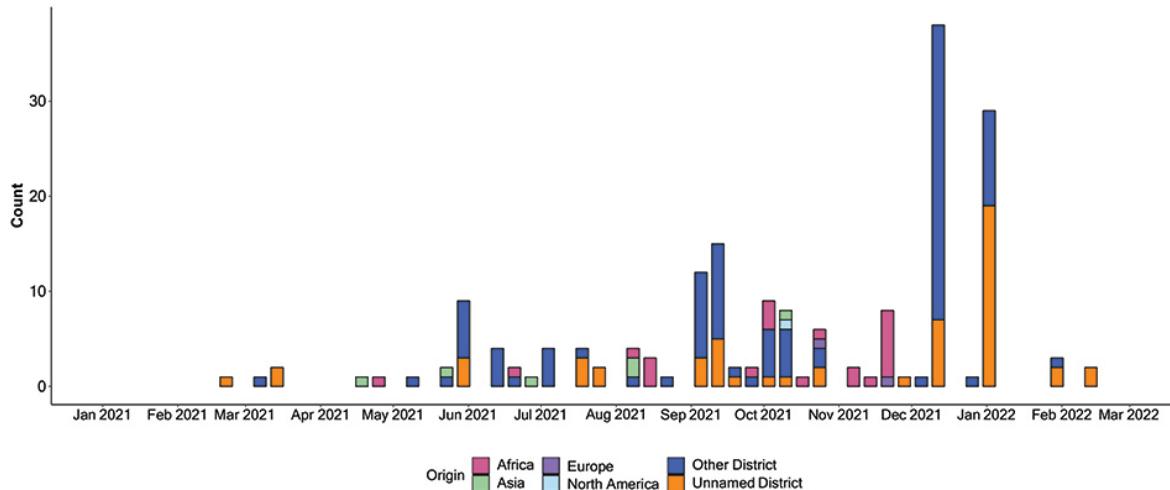
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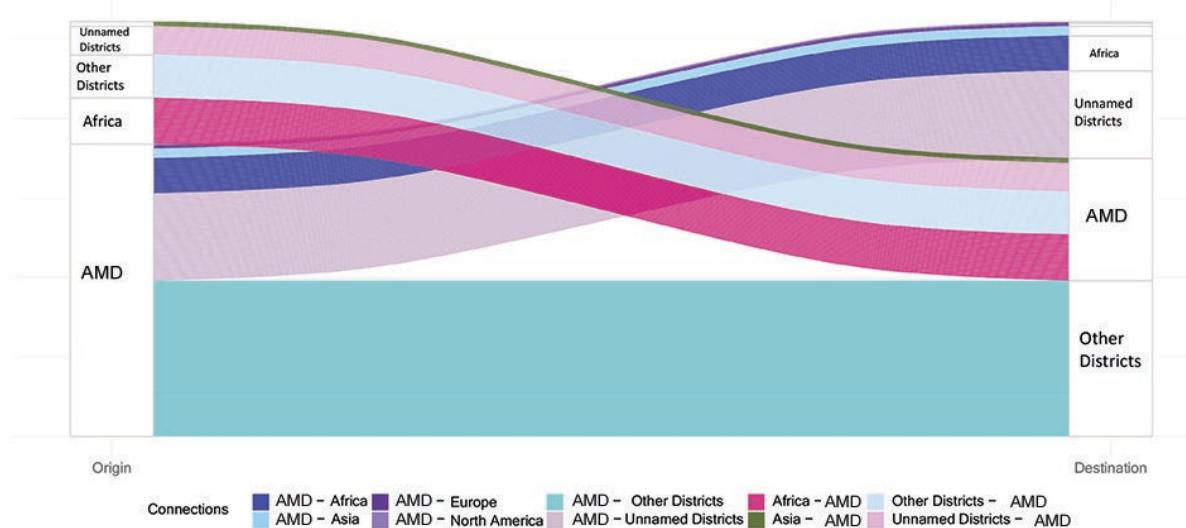
**Appendix Figure 1.** Overview of SARS-CoV-2 lineages over time by districts. Samples that came from within the Greater Accra Region but without a clear indication of the specific district have their districts indicated as 'Unnamed District'.

**A****B**

C



D



**Appendix Figure 2.** Phylogenetic relationship and spread of SARS-CoV-2 lineages in the Greater-Accra region (GAR) of Ghana. A) Rooted maximum-likelihood tree of SARS-CoV-2 variants in the GAR inferred from whole-genome sequencing data. The colors of the tips represent the SARS-CoV-2 variants showing the 5 major variants recorded; all others were classified as “Other.” The colors of the heatmap indicate the district in the GAR from which the sample was collected. Samples from returning international travelers who tested positive for COVID-19 at arrival at the Kotoka International Airport (Accra, Ghana)

are classified as “Outside Ghana.” The tree was rooted at the Wuhan reference genome (GenBank accession no. NC\_045512.2). B) SARS-CoV-2 importation events showing the number of events into the Accra Metropolitan District (AMD). C) SARS-CoV-2 exportation events showing the number of events from the AMD to other districts and regions. D) Alluvial plot showing the flow of SARS-CoV-2 importation and exportation between the regions. For panels B, C and D, 2020 data were excluded from the analysis because of paucity. The “Other District” category is combination of all the named districts excluding the AMD. For all panels, samples that came from within the GAR but without a clear indication of the specific district are indicated as “Unnamed District.”